# Working Group on Mineral Metabolism—WGMM

## Abstracts for Oral Presentations

### 229 HUMAN FETAL CARTILAGE : PRESENCE OF TWO VITAMIN D-DEPENDENT CALCIUM-BINDING PROTEINS (CaBP 28k-Da and CaBP 9k-Da)

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Both vitamin D-dependent calcium-binding proteins (28k-Da and 9k-Da) are synthetized in response to $1,25(OH)_2D_3$, the hormonal form of vitamin D. Thus, their presence in a tissue suggests a role of vitamin D in it. The immunolocalization of the two CaBPs has been studied in the proximal extremity of the tibia in the 22nd week of gestation (trisomy 21).

Tissue samples were frozen in liquid nitrogen, fixed in Carnoy and cryosectioned. The immunolocalization was performed using protein A-peroxidase with the two specific antisera:antisera raised in rabbit against rat renal 28k-Da CaBP and against rat duodenal 9k-Da CaBP. Tests included controls using non-immune serum.

In the proximal tibial extremity, both CaBPs were found exclusively in the epiphysis; the zone differentiated into a growth plate cartilage, showed non specific staining for either protein. The localization is intracellular; the cytoplasm was negative. For most chondroblasts, the specific staining is localized in the nuclear chromatin. Occasionally, in those chondroblasts having faint nuclear staining, there was intense specific staining for both CaBPs in the perinuclear area.

These findings show, for the first time, the simultaneous presence of the two CaBPs in the same cells. Their localization in chondroblasts at a stage of development prior to chondrocyte phenotype differentiation suggests a role for vitamin D in differentiation of human fetal chondroblasts; their presence in cells undergoing intense mitotic activity suggests that they are involved in chondroblast nuclear processes.

### 230 EXPRESSION OF CHOLECALCIN (9,000MW CHOLECALCIFEROL-INDUCED CaBP) GENE DURING THE DEVELOPMENT OF RAT AND HUMAN FETUSES.

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The marked rise in intestinal absorption of calcium and in the placental transfer of this ion in rats during the last days of gestation is associated with an increase in CaBP 9K in the maternal duodenum and placenta (Delorme et al. J. Dev. Physiol. 1, 181, 1979). We now report a comparative analysis of the expression of the CaBP 9K gene in the maternal duodenum and in maternal and fetal parts of the placenta at different stages of the gestation in rat and also in specific tissues during the development of human fetuses. CaBP 9K mRNA levels were examined in these tissues by Dot-blot hybridization. Size analysis of the mRNA sequences was performed by electrophoretic fractionation on agarose gels and Northern hybridization to $^{32}P$ cloned rat duodenal CaBP 9K cDNA. A homogeneous 500-600 nucleotide mRNA species was shown when RNA from different tissues was hybridized to the cDNA probe. In the rat, the highest concentration of CaBP 9K mRNA occured on the last day of gestation in both tissues. At this time, CaBP 9K mRNA was 20-30 times less concentrated in fetal and maternal parts of placenta than in the maternal duodenum. CaBP 9K mRNA concentration in the maternal duodenum doubled from day 15 to day 21 while in the maternal placenta CaBP 9K mRNA increased 7 fold during the same period. Studies in the human fetuses reveal that the CaBP 9K-Da mRNA appeared early during the development in specific tissues. This evolution of CaBP 9K mRNA levels in maternal duodenum and fetal placenta during pregnancy provides evidence for an active process of CaBP 9K biosynthesis associated with concomittant changes in calcium transfer in these tissues.

### 231 ABNORMAL SKELETAL DEVELOPMENT IN GOATS WITH CONGENITAL HYPOTHYROIDISM.

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In some infants with congenital hypothyroidism (CH) X-ray photographs of the long bones reveal increased mineral content and absence of the medullary cavity, resembling osteopetrosis. We studied this feature in goats with goitrous CH, resulting from a familial defect in the structure of thyroglobulin. Thirteen goitrous animals and 6 normal controls, 0-9 months old, were used. Decalcified routinely stained sections of the proximal femur were used for histological and histomorphometric study. Thick (100 µm) undecalcified transvere sections of the femoral midshaft were used for microradiographic investigation. In the CH-animals reconstruction of bone tissue was reduced: In normal young goats 48.7 ± 6.2 (SD)% of trabecular surfaces were covered by active osteoblasts and 5.7 ± 1.7% by osteoclasts. In the goitrous goats these figures were resp. 22.8 ± 13.5% and 2.1 ± 1.8% ($p < 0.05$). In the most severely affected animals calcified cartilage and primary bone persisted, almost obliterating the marrow cavity; resorption of metaphyseal trabeculae was virtually non-existent. The diameters of the diaphysis and the medullary cavity varied widely due to growth of the goats, but they were no different in the 2 groups.

It is concluded that in CH the condition resembling osteopetrosis results from severely delayed resorption of primary cartilage and bone, and normal development of cortical bone.

### 232 BONE MINERAL CONTENT IN APPROPRIATE AND SMALL FOR GESTATIONAL AGE NEWBORN INFANTS: A REFERENCE FOR THE EVALUATION OF POSTNATAL BONE MINERALIZATION

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Bone mineral deficiency is now a well known entity which develops predominantly in extremely low birth weight infants during the first weeks of life. Bone mineral content (BMC) can be measured by photonabsorptiometry. The aim of the study was to investigate the influence of the nutritional status on BMC and to establish a reference range for BMC. Method: BMC (mg/cm) of the right middle humerus was measured by photonabsorptiometry in 99 appropriate for gestational age (AGA) infants (birth weight ≥10th centile, range 890 - 6760 g) and in 25 small for gestational age (SGA) infants (birth weight <3rd centile, range 730 - 2550 g) within the first week of life. Linear regression analysis was performed separately for all AGA infants, 40 AGA infants (birth weight <2550 g), and all SGA infants. Results: The xy-plot showed a linear relationship between BMC and birth weight. The following regression lines were calculated. AGA (n=40): BMC (mg/cm) = 0.3989 x weight (g)+39.63. SGA: BMC = 0.0285 x weight + 57.831. The slopes of the regression lines showed no significant difference (F-test, P = 0.46), i.e. BMC of SGA infants was shown to be equivalent to that of AGA infants of the same weight. The 95 % tolerance limits of the reference range are

$$BMC = 0.4568 \times \text{weight} + 31 \pm 24.6211\sqrt{0.01 + \frac{(\text{weight} - 2798)^2}{108877407}}$$

Conclusion: Postnatal bone mineralization should be assessed by comparison with a BMC reference which takes birth weight into account rather than age.

### 233 *EVALUATION OF SERUM OSTEOCALCIN (OC) AS AN INDEX OF ALTERED BONE METABOLISM IN CHILDREN AND ADULTS*

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*Recent evidence suggests that OC is like the bone alkaline phosphatase (AP) produced by osteoblasts and circulates in human blood. With the introduction of a RIA for serum OC by Price et al. (J Clin Invest 66:878,1980) it was hoped that this test would provide a sensitive index of altered bone metabolism. We therefore measured serum OC in 88 controls and 112 patients with (1) disorders of Ca metabolism (2) isolated hyperphosphatasemia in the absence of disease (IH) and (3) children prone to osteopenia. Results: Controls: OC was higher in children <15 yr (x̄±2 SD: 8.2-14.8 ng/ml) than adults (2.5-5.5 ng/ml) and correlated with serum AP (r=0.87, n=88, p<0.01). Patients: (1) OC was high in primary hyperparathyroidism, low in untreated hypoparathyroidism (HP) but normal in HP on vit. D. OC was low-normal in rickets and increased during vit. D therapy except in patients with end organ resistance to $1,25-(OH)_2D_3$. (2) OC (and urinary hydroxproline) were not elevated in IH, indicating that other mechanisms than increased bone turnover may account for the markedly elevation of serum AP in these subjects. (3) OC was decreased in children with diabetes mellitus type I and in patients on glucocorticoid treatment, indicating decreased bone formation. Conclusion: Serum OC (1) seems to be dependent on the circulating $1,25-(OH)_2D$ levels and normal action of the vit. D hormone, (2) may be of value in the differential diagnosis of unexplained AP elevation, (3) appears to be a sensitive index of bone formation.*

### 234 EXTRA- AND INTRACELLULAR MG AND ELECTROLYTE CONCENTRATIONS DURING PREGNANCY AND AT BIRTH : FETO-MATERNAL RELATIONSHIP.

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During pregnancy, Mg deficiency may play a role in pathological states such as uteroplacental insufficiency, gestosis, premature delivery and low birth weight babies. A longitudinal study was undertaken to establish the normal values of the following parameters in maternal and mixed cord blood : plasmatic concentration of Na, K, P, Ca, Mg and protein and intracellular concentration of Na, K, Mg in lymphocytes and erythrocytes. During pregnancy, the previously described fall of total serum Mg concentration was found. The mean intracellular Mg however did not change significantly. However, in 10/35 women, intraerythrocyte and lymphocyte Mg concentration had a tendency to fall. At birth and for every studied extracellular ion but Na, there was a concentration gradient mother-cord indicating the role of the placenta in ionic transfers. In erythrocytes, there was a significant correlation between mother and cord blood for each ion. In lymphocytes, this correlation was only present for Mg. Furthermore, there was a constant correlation between Mg and K concentration in both erythrocytes and lymphocytes. This could be related to effect of Mg ions on cell membranes and/or the Na/K ATPase transport system.